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# Reliability of flow-mediated dilation measures in the popliteal artery and implications for use in clinical and research practices

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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By

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THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

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## ABSTRACT

**PURPOSE:** The aim of the present study was to assess the test-retest and stability reliability of flow-mediated dilation (FMD) in the popliteal artery and to investigate the effect of occlusion pressure on the FMD response. **METHODS:** A series of FMD tests were performed on ten healthy young adult males to assess reliability. Ultrasound-derived artery diameter of the popliteal was measured and FMD was calculated as the percent change in diameter from baseline. **RESULTS:** FMD measurements for intra- and interday comparisons demonstrated poor reliability (Repeatability 5.62 and 4.82%, Intraclass correlation coefficient [ICC] 0.36 and 0.25, respectively). Repeatability values were as large as the FMD measures themselves for both intra- and interday reliability. **CONCLUSION:** Popliteal artery FMD has poor reliability for test-retest and stability reliability. Interpretation of individual or group changes using this technique should be interpreted with caution.



**Keywords:** Flow-mediated dilation, Endothelial function, Reliability, Popliteal artery

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## LIST OF ABBREVIATIONS

CV – Coefficient of variation

CVD – Cardiovascular Disease

$Ca^{++}$ – Calcium

ECG - Electrocardiography

EDHF – Endothelial-derived relaxing factor

eNOS – Endothelial nitric oxide synthase

FMD – Flow-mediated dilation

ICC – Intraclass correlation coefficient

L-NMMA – $N^{G}$-monomethyl-L-arginine

MBV – Mean blood velocity

NO – Nitric oxide

PGs - Prostaglandins

SD – Standard deviation

Sw – Within subject standard deviation

# Chapter 1:

# Review of Literature

## 1.1 Introduction

When blood flow increases through a blood vessel, a resultant frictional force parallel to the vessel, termed shear stress, is applied. An increase in shear stress on the vascular endothelium causes endothelium-dependent vasodilation (flow-mediated vasodilation [FMD]). In 1992, Celermajer et al. (Celermajer *et al.*, 1992) developed a technique that took advantage of this physiological response and is currently a widely used, noninvasive technique to provide insight into vascular health.

The reactive hyperemia endothelial function test, commonly referred to as an FMD test, uses ultrasound assessment of FMD in response to cuff release following an occlusion period. This technique generates a shear stress stimulus, resulting in a dilation of downstream resistance vessels following the occlusion of blood flow to the limb with a pressure cuff. Upon release of the occlusion, inflow through the conduit artery is transiently increased (reactive hyperemia) and acts as the stimulus for FMD.

In humans FMD is typically assessed in the large peripheral conduit arteries and is considered representative of the response in more clinically relevant coronary circulation (Anderson *et al.*, 1995; Takase *et al.*, 1998). As a result of the close relationship to the coronary circulation, the FMD test has become widely used as a measure of endothelial dysfunction in both clinical and asymptomatic patients. Experimental and clinical studies suggest that endothelial dysfunction is an important feature of vascular disease and is strongly associated with several cardiovascular conditions, including atherosclerosis (Ross, 1999), hypertension (Taddei *et al.*, 1993; Perticone *et al.*, 2001; Modena *et al.*, 2002), and coronary and peripheral artery disease (Yataco *et al.*, 1999; Zhang *et al.*, 2000; Neunteufl *et al.*, 2000; Kuvin *et al.*, 2001;

Brevetti *et al.*, 2003), and that endothelial dysfunction predicts cardiovascular events in these groups (Gokce *et al.*, 2002; Widlansky *et al.*, 2003).

## 1.2 Physiology of FMD

The endothelium is a single layer of cells lining all of the blood vessels in the body, also known as the tunica intima, and has been identified as playing a major role in smooth muscle dilation. Animal studies established that FMD in arteries was dependent on the presence of an intact endothelial lining (Smiesko *et al.*, 1985; Pohl *et al.*, 1986). Rubanyi et al. (Rubanyi *et al.*, 1986) indicated that, in response to shear stress, the endothelium released a substance that possessed the characteristics of Furchott's endothelium-derived relaxing factor, now known as nitric oxide (NO) (Moncada *et al.*, 1988).

Reductions in FMD are widely assumed to reflect diminished NO production as several pivotal human studies have concluded that brachial and radial artery FMD are dependent on an NO pathway (Joannides *et al.*, 1995; Mullen *et al.*, 2001; Doshi *et al.*, 2001; Jiang *et al.*, 2011). Studies involving the administration of NO blockades, such as $N^G$-monomethyl-L-arginine (L-NMMA), have confirmed the major role that NO plays in vasoregulation. Joannides et al. (Joannides *et al.*, 1995) found that radial artery dilation following 3 minutes of ischemia was abolished in the presence of L-NMMA. Similarly, Mullen et al. (Mullen *et al.*, 2001) found that NO blockade decreased the radial artery FMD response to 5 minutes of ischemia from 5.3% to 0.7% dilation, with no difference in hyperemic stimulus, concluding that it was unlikely that stimulus magnitude was responsible for the abolished FMD response.

Figure 1 outlines the pathway of endothelial-dependent vasodilation in response to increases in fluid shear stress. Although several vasodilators are released by the endothelium, the FMD test typically tries to isolate the NO pathway. Following release of occlusion, during

an FMD test, the resultant increase in blood flow creates a shear stress stimulus causing deformation of mechanosensitive structures on the endothelial cell membrane, such as membrane proteins (glycocalyx), primary cilia and mechanosensitive ion channels (Pyke & Tschakovsky, 2005; Davies, 2009). The acute response to the shear stress stimulus is the opening of calcium ($Ca^{++}$)-activated potassium channels, causing hyperpolarization of the endothelial cell (Olesen *et al.*, 1988; Cooke *et al.*, 1991; Miura *et al.*, 2001). This results in an increased driving force for $Ca^{++}$ entry into the cell. The $Ca^{++}$ activates endothelial nitric oxide synthase (eNOS) which in turn increases the conversion of L-arginine to NO (Pohl *et al.*, 1986; Joannides *et al.*, 1995). Over prolonged periods of shear stress stimulus (minutes), the mechanosensitive structures signal increases G-protein expression and resultant phosphorylation of eNOS (Corson *et al.*, 1996; Dimmeler *et al.*, 1999). The increase in eNOS activity increases the production of NO, even at low concentrations of calcium. Vasodilators diffuse from the endothelial cell into the tunica media (composed of smooth muscle), trigger a signalling cascade which ultimately reduces intracellular [$Ca^{++}$] and induces relaxation of the smooth muscle and subsequent vasodilation.

As mentioned previously, there is some redundancy in the mechanisms controlling vasodilation. In addition to multiple mechanosensitive structures on the endothelial cell surface, there are also multiple vasodilatory pathways within the endothelial cell which may facilitate FMD (Sun *et al.*, 1999; Mullen *et al.*, 2001; Doshi *et al.*, 2001; Pyke *et al.*, 2009; Parker *et al.*, 2011). A study using blood vessels from mice genetically engineered to lack eNOS still responded to shear stress by dilating (Sun *et al.*, 1999). Other animal models have confirmed prostaglandins (PGs) and endothelial-derived hyperpolarizing factor (EDHF) also contribute to endothelium dependent vasorelaxation (Huang *et al.*, 1998; Pak *et al.*, 2002; Scotland *et al.*, 2005). More recently Pyke et al. (Pyke *et al.*, 2009) were unable to reduce radial artery FMD

with a large dose of L-NMMA and concluded that there may be heterogeneous vasodilator phenotypes which affect the contribution of NO to FMD.

## 1.3 Methodological Considerations

FMD has emerged as a popular technique in both clinical and physiological studies to examine the mechanisms that impact endothelial and vascular function. It is clear, that minor changes in the methodological approach can significantly alter the nature and magnitude of the FMD response. As a result of this, a series of reviews and tutorials have been published in an attempt to standardize the protocol of this widespread technique (Corretti *et al.*, 2002; Pyke & Tschakovsky, 2005; Harris *et al.*, 2010; Thijssen *et al.*, 2011*a*). Most recently, Thijssen et al. (Thijssen *et al.*, 2011*a*) published methodological and physiological guidelines for several key aspects of the assessment of FMD in humans. These guidelines included recommendations regarding subject preparation, test protocol, Doppler ultrasound technique and data analysis.

FMD is typically assessed in peripheral conduit arteries, such as the brachial, radial and superficial femoral, although the popliteal artery has recently emerged as an alternative site for assessing endothelial function in the lower limbs. During an FMD test, baseline ultrasound-derived artery diameter and Doppler mean blood velocity (MBV) is established prior to the occlusion period. Once baseline measures have been taken a pressure cuff is inflated to occlude blood flow to the forearm or lower leg, depending on the location of the FMD test. Cuff position (distal or proximal to ultrasound measurements) has been shown to alter the FMD response by altering the contribution of vasoactive substances (Uehata *et al.*, 1997; Mannion *et al.*, 1998; Vogel *et al.*, 2000). Distal cuff occlusion on the brachial artery was associated with a 7% FMD, which was abolished by administration of NO blockade (Doshi *et al.*, 2001). Alternatively, proximal cuff occlusion was associated with a 12% brachial artery FMD that was only partially

reduced (to 7.5%) with the administration of an NO blockade. These data suggest that cuff placement may affect the nature of the FMD response, by influencing the heterogeneity of vasodilatory pathways in the endothelium. Distal cuff occlusion is considered to be greatly NO-mediated, whereas additional factors are contributing to the dilation associated with proximal cuff placement. Consequently, distal cuff placement is acknowledged as the standard practice to elicit an NO-dependent response.

In addition to cuff position, occlusion duration can also affect the FMD response. The change in brachial FMD increases as the duration of cuff inflation increases from 30 seconds to 5 minutes (Corretti *et al.*, 2002). Although it has been suggested that there is no change in dilation following 5 or 10 minutes of occlusion (Corretti *et al.*, 2002), this remains unclear. Some studies have found that a 10 minute occlusion period results in a greater FMD response that is a result of contributions from non-NO, ischemic-induced vasodilators (Kooijman *et al.*, 2008; Harris *et al.*, 2009). As a result, a 5 minute occlusion period is the accepted duration to mediate an NO-dependent FMD response.

Following the 5 minutes of distal cuff occlusion, diameter and MBV are monitored for at least 3 minutes in upper limb arteries and 5 minutes in lower limb arteries. Studies have found that peak diameters in the brachial artery, and similarly in the radial, typically occur within the first 120 seconds following release (Black *et al.*, 2008; Irace *et al.*, 2008; Padilla *et al.*, 2009; Liuni *et al.*, 2010). Arteries in the legs demonstrate a significantly later peak than those in the arms (Thijssen *et al.*, 2008). Therefore, arteries such as the superficial femoral and popliteal should be monitored for 5 minutes following cuff release to ensure adequate detection of peak diameter.

## 1.4 Rationale

The magnitude of FMD of the conduit arteries is a widely used test of endothelial function. The FMD test has been documented to correlate with invasively assessed endothelial function in the coronary arteries (Anderson *et al.*, 1995), and is now commonly used as an index of endothelial function and is often applied as a surrogate marker of cardiovascular disease (CVD). As a diagnostic tool, it is important to understand the repeatability of this measure and the test-retest reliability, or the ability to reproduce similar results from consecutive tests within a single day. It is critical to comprehend the limitations, if there are any, to taking a single measure compared to an average of multiple FMD tests. Additionally, due to the technique's application in tracking changes in pre-post interventions, it is important to understand the accuracy of repeating measurements on separate days, and what percentage of changes is due to measurement error compared to a physiological adaptation.

Studies in the brachial (Welsch *et al.*, 2002; Peretz *et al.*, 2007; Magda *et al.*, 2012) and radial arteries (Brook *et al.*, 2005) have found conflicting results concerning the reliability of the FMD test. Some studies have found that the FMD technique is a stable and reproducible marker of vascular function in the brachial artery (Welsch *et al.*, 2002). Alternatively, other studies have shown that brachial FMD may only be satisfactory (Magda *et al.*, 2012), or in the case of the radial artery, a very poor (Brook *et al.*, 2005) indicator of vascular function due to high variability between repeated measures. We are unaware of any studies detailing acceptable values for day-to-day variability and test-to-test repeatability within the ultrasound measure of FMD of the popliteal artery. Due to the increasing use of this noninvasive technique in clinical settings as a prediction of CVD risk, and in physiological research as an assessment of endothelial function, there is a strong need for stability of this measure. The present study aims

to examine the test-retest reliability (variability between repeated trials within a day) and stability reliability (day-to-day variability in measurements) of the FMD measurement in the popliteal artery.

Between-laboratory comparisons of the magnitude of FMD are often made difficult due to the use of different experimental protocols that may affect the physiological response. The use of different occlusion pressures is a major aspect of FMD protocol that is inconsistent between laboratories, and the effect on the physiological response of the endothelium is unclear. Theoretically any pressure above resting systolic pressure (suprasystolic) should be sufficient to occlude blood flow however little research has been done to look at the effects of different pressures used on the FMD response. The present study will permit insight regarding the effect of different occlusion pressures on the FMD response.

Overall, the main objectives of the study were 1) to assess the test-to-test and day-to-day reliability of flow-mediated dilation in the popliteal artery; and 2) to examine the effects of five different occlusion pressures on the flow-mediated dilation response in the popliteal artery. We hypothesized that 1) there will be good test-retest and stability reliability of flow-mediated dilation between tests repeated within the same day and on separate days; and 2) there will be no difference in flow-mediated dilation with the five different occlusion pressures.

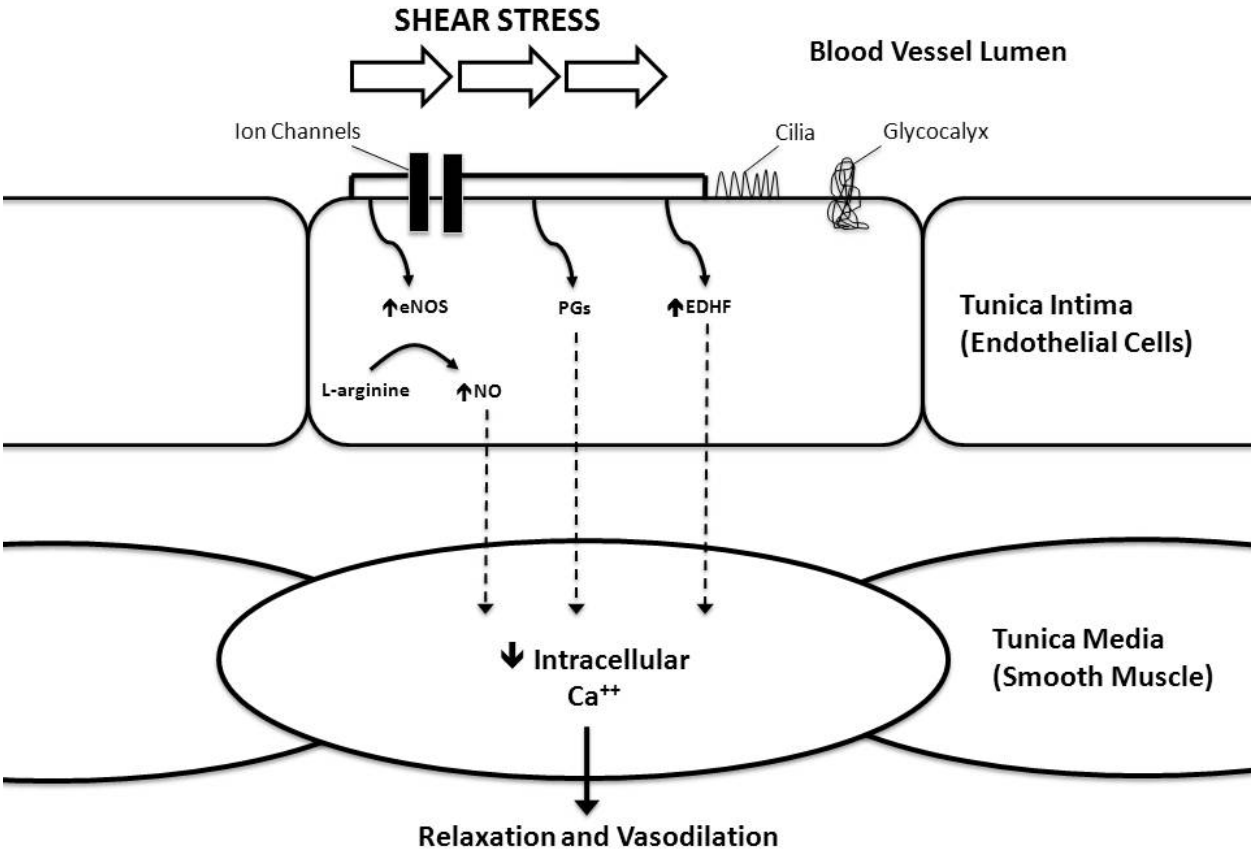



Fig. 1.1 Schematic representation of the pathways involved in flow-mediated dilation (FMD) from the initial fluid shear stress stimulus to the resultant change in vessel diameter.

# Chapter 2:

## Reliability of flow-mediated dilation measures in the popliteal artery and implications for use in clinical and research practices

## 2.1 INTRODUCTION

Flow-mediated dilation (FMD) describes the vasodilation of a conduit artery following an increase in shear stress. This is typically induced by a five minute period of ischemia generated by an inflated cuff placed distal to the artery of interest (Corretti *et al.*, 2002). The FMD response is largely nitric oxide (NO)-mediated (Joannides *et al.*, 1995; Mullen *et al.*, 2001) and provides information about the integrity of the endothelium (Vita & Keaney, 2002). Studies have shown that impaired endothelial vasodilation is an important feature of vascular disease and is strongly associated with several chronic cardiovascular conditions (Neunteufl *et al.*, 2000; Perticone *et al.*, 2001; Kuvin *et al.*, 2001; Gokce *et al.*, 2002; Modena *et al.*, 2002; Widlansky *et al.*, 2003). The reactive hyperemia endothelial function test, commonly referred to as an FMD test, is presently a widely used, noninvasive technique to provide insight into peripheral conduit artery vasoreactivity.

The FMD technique has increasingly been applied in both clinical and physiological studies to examine the mechanisms that impact endothelial and vascular function. FMD is typically assessed in the peripheral conduit arteries, such as the brachial, radial and superficial femoral. More recently, the popliteal artery has emerged as an alternative location for assessing endothelial function in the lower limbs. The magnitude of FMD in the conduit arteries is a widely used test of endothelial function. The FMD test has been documented to correlate with invasively assessed endothelial function in the coronary arteries (Anderson *et al.*, 1995), and is now commonly used as an index of endothelial function and a surrogate marker of cardiovascular disease (CVD). As a diagnostic tool, it is important to understand the test-retest reliability, or the ability to reproduce similar results from consecutive tests within a single day, to determine if one FMD test is adequate to represent vasoreactivity of the artery. Additionally it is

important to understand the accuracy of repeating measurements on separate days, and what percentage of changes is due to measurement error instead of physiological adaptation.

Studies in the brachial (Welsch *et al.*, 2002; Peretz *et al.*, 2007; Magda *et al.*, 2012) and radial arteries (Brook *et al.*, 2005) have found conflicting results concerning the reliability of the FMD test. Some studies have found that the FMD technique is a stable and reproducible marker of vascular function (Welsch *et al.*, 2002). Alternatively, other studies have shown that FMD may only be satisfactory (Magda *et al.*, 2012), or a very poor (Brook *et al.*, 2005) indicator of vascular function due to high variability between repeated measures. We are unaware of any studies detailing acceptable values for day-to-day variability and test-to-test repeatability within the ultrasound measure of FMD in the popliteal artery. With the increasing use of this noninvasive technique in clinical settings to predict risk of CVD, and in physiological research as an assessment of endothelial function, there is a strong need for stability of this measure. The present study aims to examine the test-retest reliability (variability between repeated tests within a single day) and stability reliability (day-to-day variability in measurements) of the FMD measurement in the popliteal artery.

Additionally, between-laboratory comparisons of the magnitude of FMD are often difficult to make because of different experimental protocols which may affect the physiological response. The use of different occlusion pressures is a major aspect of FMD protocol that remains inconsistent between laboratories, and the effect on the physiological response is unclear. Theoretically any occlusion pressure above resting systolic pressure (suprasystolic) should be sufficient to occlude blood flow however little research has been done to look at the effects of different pressures used on the FMD response. The present study will permit insight regarding the effect of different occlusion pressures on the FMD response.

Overall, the main objectives of the study were 1) to investigate the day-to-day and test-to-test reliability of flow-mediated dilation in the popliteal artery; and 2) to examine the effects of five different occlusion pressures on flow-mediated dilation responses in the popliteal artery. We hypothesized that 1) there will be good test-retest and stability reliability of FMD between tests repeated within the same day and on separate days; and 2) there will be no difference in FMD with the five different occlusion pressures.

## 2.2 METHODS

### 2.2.1 Participants

Ten healthy young men (27 ± 6 yr; mean ± SD; Table 2.1) volunteered and gave written consent to participate in the study. All procedures were approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects. All participants were recreationally active (regularly exercising to maintain fitness) and non-smokers. Additionally, all subjects were normotensive and no subjects were taking medications that would affect hemodynamic responses.

### 2.2.2 Study Design

A series of FMD tests were performed on each participant over five consecutive days (Figure 2.1). Tests were performed at the same time each day to minimize diurnal effects. Two FMD tests were performed on each of four days, with a fifth day involving three FMD tests. Each FMD test was separated by a 30 minute rest period to allow blood flow and arterial dilation to return to resting conditions (Harris *et al.*, 2006). The first FMD test of each day was performed with a different occlusion pressure found throughout the literature: 175; 200; 225; 250; and 300 mmHg. The succession of the first FMD test occlusion pressure was randomized between subjects, such that one subject may have an occlusion pressure of 175 mmHg for their first test on day 1, while another subject may have an occlusion pressure of 250 mmHg. The second FMD test of each day was performed with an occlusion pressure of 250 mmHg, resulting in five tests performed in five consecutive days at the same pressure – allowing analysis regarding day-to-day variation. On the day when two FMD tests were performed with an

occlusion pressure of 250 mmHg, a third test at that pressure was performed allowing determination of the test-retest reliability for three tests within the same day.

### 2.2.3 Protocol

FMD of the popliteal artery was assessed in accordance with previously published guidelines for the current standardized methodology (Corretti *et al.*, 2002; Thijssen *et al.*, 2011*a*). All participants were instructed to refrain from caffeine, alcohol and exercise for 12 hours prior to their scheduled appointments. Following at least 10 minutes of supine rest, participants were instructed to lie prone and a small pillow was placed under their ankle. The left popliteal artery was measured immediately proximal to the bifurcation (usually at or slightly above the popliteal fossa), and a pneumatic cuff (Flexiport; Welch Allyn Inc., Skaneateles Falls, NY, USA) was placed around the calf (approximately 2-3 inches distal to the popliteal fossa). Heart rate was continuously monitored with a three-lead ECG to allow for consistent and accurate selection of arterial diameter measurements during the cardiac cycle.

The popliteal artery was imaged with a 10-MHz multifrequency linear-array transducer attached to a Doppler ultrasound machine (VingMed System FiVe, GE Medical Systems, Horten, Norway). All scans were performed by an experienced investigator with training obtained through pilot studies, other research projects and comparisons to a phantom artery to insure accurate assessment of arterial diameters. All scans were made under similar conditions and all images were recorded on an external video camera (HDD Everio; JVC, Canada) for later offline analysis. Baseline diameter was recorded prior to manual inflation of the pneumatic cuff. The cuff was then inflated to the predetermined occlusion pressure, according to which test in the series was being performed. The cuff was inflated for 5 minutes during which diameter was not recorded. 15 seconds prior to release of the cuff the video camera started recording. At exactly

5 minutes the pneumatic cuff was released and arterial diameter was continuously monitored for 5 minutes post-release (Figure 2.2).

### 2.2.4 Popliteal Artery Diameter Analysis

All videos were uploaded and then analyzed using a software program (VirtualDub 1.6.19.0 by Avery Lee) which allows for frame by frame analysis to ensure that diameter measurements were always taken at end diastole (determined by ECG gating). Triplicate measurements of diameter were taken for each of five baseline images and averaged to determine the baseline diameter of the artery. Similarly, triplicate measurements of diameter were averaged for a single image taken every 15 seconds following cuff release. Diameter measurements were defined as the distance between the media and intima interface of the near wall and far wall (see Figure 2.3). Peak diameter was determined as the post-occlusion image with the largest diameter and FMD was then calculated as the percent change in diameter from resting baseline. Delta was calculated as the difference between peak and baseline diameters [Delta (mm) = Peak diameter – Baseline diameter].

### 2.2.5 Statistical Analysis

All statistical analyses were performed using SPSS software, version 19 (SPSS Inc., Chicago, Ill., USA) and Microsoft Excel 2010 (Microsoft, Seattle, Wash., USA).

Group mean, standard deviation (SD) and coefficient of variation (SD/mean) were calculated for four variables (%FMD, Baseline diameter, Peak diameter and Delta) for each test. A one-way repeated measures analysis of variance (ANOVA) was used to determine if there were significant differences within the four variables for three comparisons: the FMD tests performed at different pressures; the five FMD tests performed over consecutive days; and the three FMD tests performed within the same day. The repeatability of each variable for the three

comparisons was calculated by multiplying the within-subject standard deviation (Sw) by 2.77 [or (1.96 × √2) × Sw] (Bland & Altman, 1996). The difference between two or more measurements for the same subject is less than 2.77 × measurement error for 95% of observations (Bland & Altman, 1996). $P < 0.05$ was considered significant. Reliability of three FMD tests repeated in a single day and the five tests performed over consecutive days were assessed using the intraclass correlation coefficient ($ICC_{(1,1)}$), which was based on the repeated measures ANOVA with testing session as the independent variable (Shrout & Fleiss, 1979).

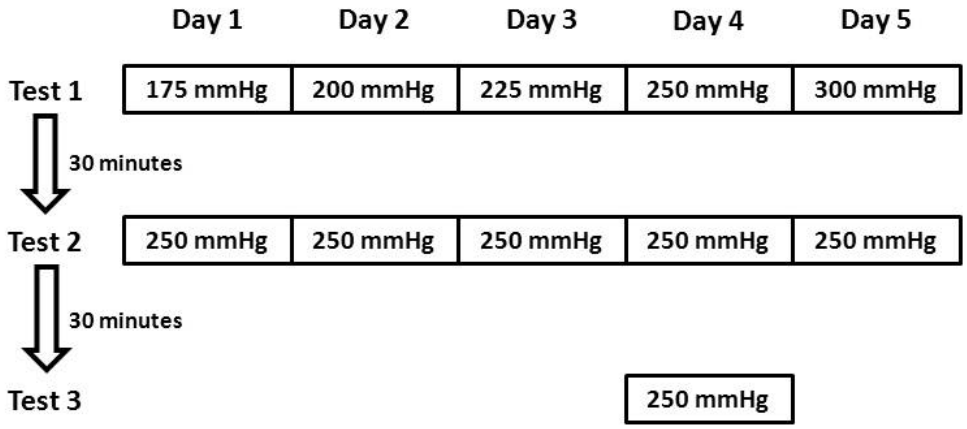



**Figure 2.1** Study design outlining the series of FMD tests. The succession of the occlusion pressure for test one was randomized between subjects.

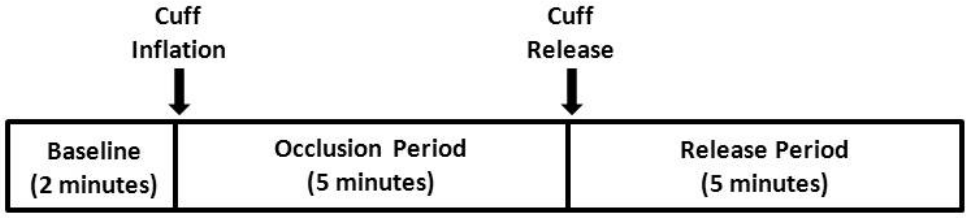
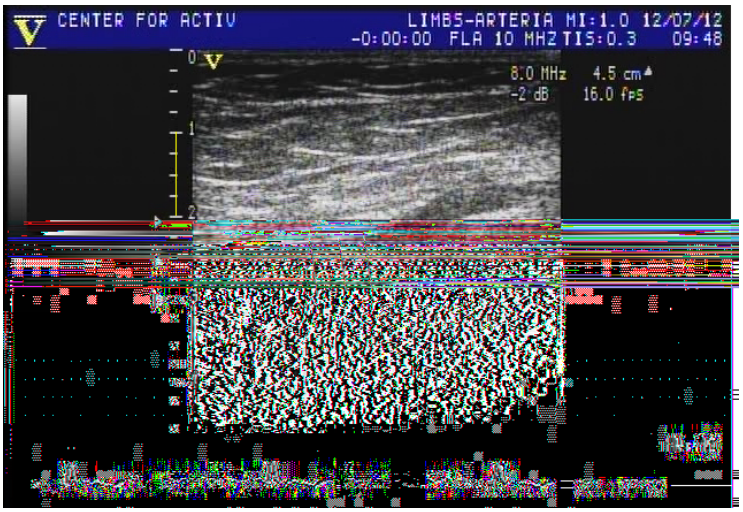



**Figure 2.2** Schematic representation of the timeline for the FMD test.

A

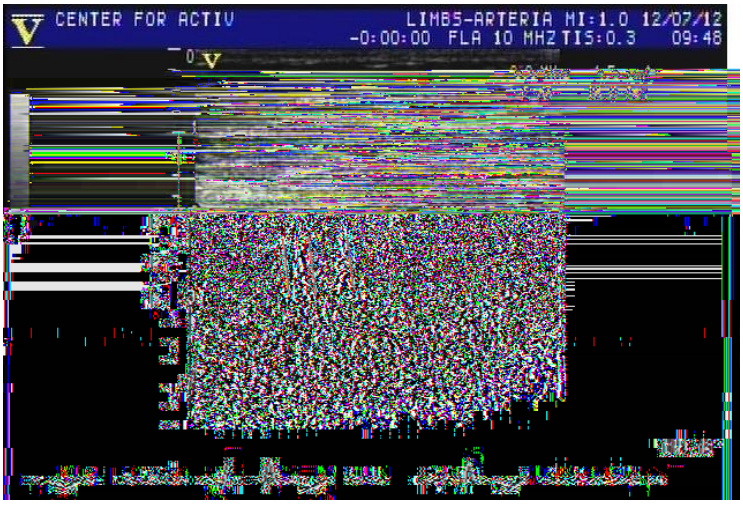



Baseline Image

Triplicate measurements

6.41 mm
6.41 mm
6.39 mm
} Averaged = **6.40 mm**

B




Peak Image

Triplicate measurements:

6.77 mm
6.77 mm
6.77 mm
} Averaged = **6.77 mm**

% dilation (FMD)

$$= \frac{\text{Peak} - \text{Baseline diameter}}{\text{Baseline diameter}} \times 100$$

$$= \frac{6.77 - 6.40}{6.40} \times 100$$

= **5.8 % dilation**

**Figure 2.3** Example calculation of baseline diameter (A) and peak diameter and FMD (B) for a representative subject.

## 2.3 RESULTS

### 2.3.1 Subject Characteristics

Subject characteristics are listed in Table 2.1.

### 2.3.2 Test-retest Reliability

The means, SDs and coefficient of variation for FMD, baseline diameter, peak diameter and delta of the three tests performed are listed in Table 2.2. There was no significant difference between the three repeats within a day at 250 mmHg occlusion pressure for any of the four variables ($p > 0.05$). The repeatability of the three FMD tests is also listed in Table 2.2. The repeatability represents the critical value at which a measurable change is observed in a given participant between tests. The repeatability of FMD was 5.62%, which means that a percent change in arterial diameter would have to be greater than 5.62 to be considered a physiological adaptation rather than measurement error. The repeatability of baseline diameter, peak diameter and delta were 1.92 mm, 2.33 mm and 0.36 mm respectively. ICCs (also listed in Table 2.2) for FMD, baseline diameter, peak diameter and delta were 0.36, 0.91, 0.86 and 0.11 respectively. The high ICCs for baseline and peak diameter indicates that there is a good reliability for those measures between tests. Alternatively the low ICCs for FMD and delta indicates that those measures have a very poor reliability over the three tests. This is also illustrated in Figure 2.4; the baseline (B) and peak diameter (C) show more consistent measurements over the three FMD tests performed whereas FMD (A) and delta (D) show much more variability between tests.

### 2.3.3 Day-to-day reliability

The means, SDs and coefficient of variation for FMD, baseline diameter, peak diameter and delta of the five FMD tests performed on separate days (ie. all at 250 mmHg occlusion

pressure) are listed in Table 2.3. There was no significant difference between the five tests for any of the four variables ($p > 0.05$). The repeatability of the five tests is also listed in Table 2.3. The repeatability of FMD, baseline diameter, peak diameter and delta were 4.82%, 1.00 mm, 1.32 mm and 0.33 mm respectively. ICCs (also listed in Table 2.3) for FMD, baseline diameter, peak diameter and delta were 0.25, 0.62, 0.52 and 0.11 respectively. Similar to the trends observed with the test-retest reliability, the day-to-day reliability of baseline and peak diameter measurements were stronger than that of FMD and delta. Again this is illustrated in Figure 2.5 depicting greater variability between subjects with FMD (A) and delta (D) compared to baseline (B) and peak (C).

### 2.3.4 Different Occlusion Pressures

The means, SDs and coefficient of variation for FMD, baseline diameter, peak diameter and delta of the five FMD tests performed at different occlusion pressures are listed in Table 2.4. There was no significant difference between the FMD tests performed at different pressures for any of the four variables ($p>0.05$). The repeatability of the five FMD tests for each of the variables is also listed in Table 2.4. The repeatability of the five FMD tests for FMD, baseline diameter, peak diameter and delta were 5.78, 1.16, 1.25 and 0.40 respectively. Figure 2.6 illustrates the variability of subjects across the different occlusion pressures. Similar to the test-retest and day-to-day reliability, there appears to be less variation with baseline (B) and peak diameter (C) measurements compared to FMD (A) and delta (D).

### 2.3.5 Time Course of Popliteal FMD

Pooling all the popliteal FMD tests together (a total of 110 tests), we were able to describe the time course of the average FMD response of the popliteal artery over 15 second intervals (shown in Figure 2.7). Time to peak FMD (6.8%) was 180 seconds post cuff release.

**Table 2.1** Subject Characteristics (n=10)

| | Age (yrs) | Mass (kg) | Height (cm) | Resting Blood Pressure (mmHg) | |
|---|---|---|---|---|---|
| | | | | Systolic | Diastolic |
| Mean | 27 | 81 | 179 | 120 | 65 |
| SD | 6 | 8 | 7 | 7 | 7 |

**Table 2.2** Test-retest reliability statistics for flow-mediated dilation properties.

| | Within Day Test | | |
|---|---|---|---|
| FMD (%) | Test 1 | Test 2 | Test 3 |
| Mean | 4.5 | 3.1 | 5.6 |
| SD | 2.5 | 2.0 | 3.5 |
| Coefficient of Variation | 0.55 | 0.64 | 0.62 |
| Between Measurement (*p* value) | - | 0.08 | - |
| Observed Power | - | 0.45 | - |
| Repeatability | - | 5.62 | - |
| Intraclass Correlation Coefficient | - | 0.36 | - |
| Baseline diameter (mm) | Test 1 | Test 2 | Test 3 |
| Mean | 6.5 | 6.7 | 6.6 |
| SD | 0.7 | 0.7 | 0.8 |
| Coefficient of Variation | 0.11 | 0.11 | 0.12 |
| Between Measurement (*p* value) | - | 0.46 | - |
| Observed Power | - | 0.16 | - |
| Repeatability | - | 1.92 | - |
| Intraclass Correlation Coefficient | - | 0.91 | - |
| Peak diameter (mm) | Test 1 | Test 2 | Test 3 |
| Mean | 6.8 | 6.9 | 6.9 |
| SD | 0.7 | 0.6 | 0.8 |
| Coefficient of Variation | 0.10 | 0.09 | 0.11 |
| Between Measurement (*p* value) | - | 0.65 | - |
| Observed Power | - | 0.11 | - |
| Repeatability | - | 2.33 | - |
| Intraclass Correlation Coefficient | - | 0.86 | - |
| Delta (mm) | Test 1 | Test 2 | Test 3 |
| Mean | 0.3 | 0.2 | 0.4 |
| SD | 0.1 | 0.1 | 0.2 |
| Coefficient of Variation | 0.48 | 0.44 | 0.59 |
| Between Measurement (*p* value) | - | 0.06 | - |
| Observed Power | - | 0.49 | - |
| Repeatability | - | 0.36 | - |
| Intraclass Correlation Coefficient | - | 0.11 | - |

- Between measurement, Observed Power, Repeatability and Intraclass Correlation Coefficient are reliability statistics that apply to all tests.

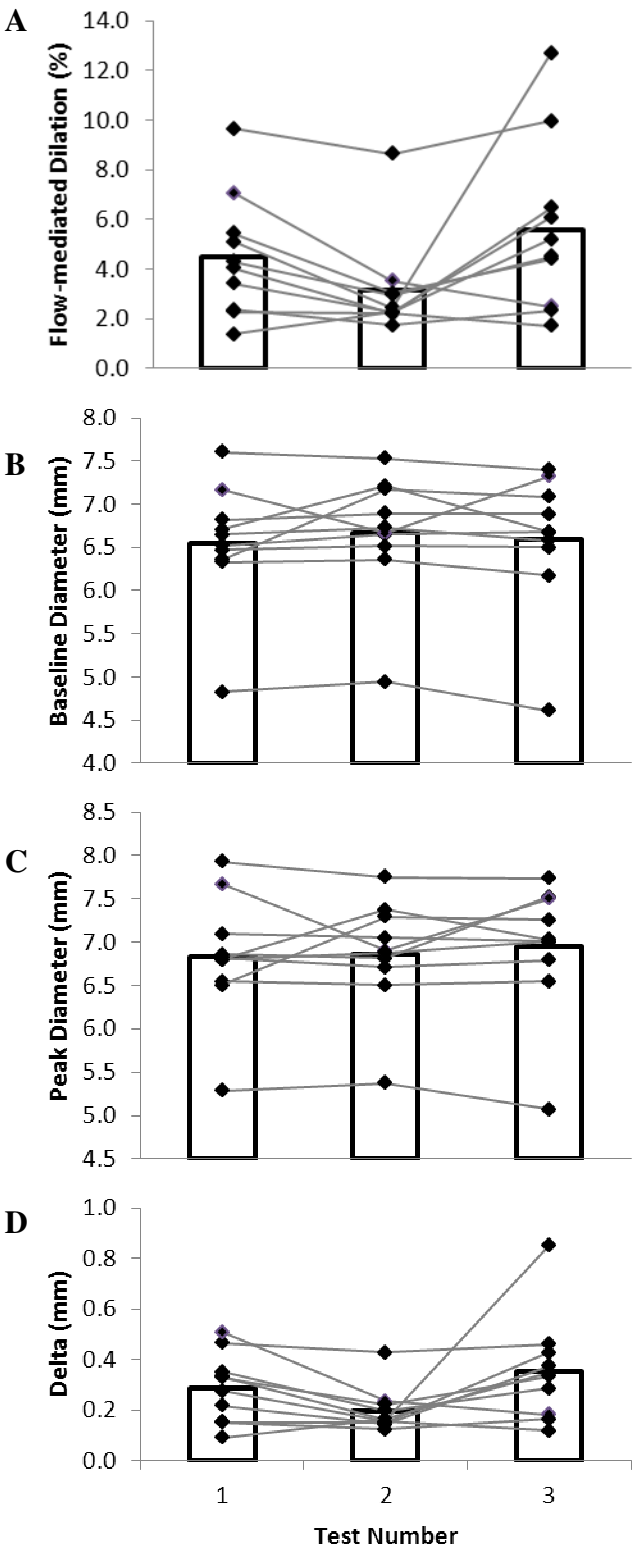



**Figure 2.4** Variability of measures between three FMD tests performed on the same day for 10 subjects. Group mean for each test is represented by the bar graph.

**Table 2.3** Day-to-day reliability statistics for flow-mediated dilation properties.

| | Day-to-Day Tests | | | | |
|---|---|---|---|---|---|
| FMD (%) | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
| Mean | 3.3 | 3.5 | 4.8 | 4.5 | 4.5 |
| SD | 2.1 | 1.6 | 1.8 | 2.3 | 2.0 |
| Coefficient of Variation | 0.64 | 0.45 | 0.37 | 0.52 | 0.45 |
| Between Measurement (*p* value) | - | - | 0.28 | - | - |
| Observed Power | - | - | 0.28 | - | - |
| Repeatability | - | - | 4.82 | - | - |
| Intraclass Correlation Coefficient | - | - | 0.25 | - | - |
| Baseline diameter (mm) | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
| Mean | 6.7 | 6.7 | 6.7 | 6.5 | 6.7 |
| SD | 0.7 | 0.5 | 0.6 | 0.5 | 0.5 |
| Coefficient of Variation | 0.11 | 0.08 | 0.09 | 0.08 | 0.07 |
| Between Measurement (*p* value) | - | - | 0.79 | - | - |
| Observed Power | - | - | 0.10 | - | - |
| Repeatability | - | - | 1.00 | - | - |
| Intraclass Correlation Coefficient | - | - | 0.62 | - | - |
| Peak diameter (mm) | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
| Mean | 6.9 | 6.9 | 7.0 | 6.8 | 7.0 |
| SD | 0.6 | 0.6 | 0.6 | 0.5 | 0.4 |
| Coefficient of Variation | 0.09 | 0.08 | 0.08 | 0.08 | 0.06 |
| Between Measurement (*p* value) | - | - | 0.73 | - | - |
| Observed Power | - | - | 0.11 | - | - |
| Repeatability | - | - | 1.32 | - | - |
| Intraclass Correlation Coefficient | - | - | 0.52 | - | - |
| Delta (mm) | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
| Mean | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 |
| SD | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 |
| Coefficient of Variation | 0.44 | 0.52 | 0.22 | 0.62 | 0.42 |
| Between Measurement (*p* value) | - | - | 0.28 | - | - |
| Observed Power | - | - | 0.25 | - | - |
| Repeatability | - | - | 0.33 | - | - |
| Intraclass Correlation Coefficient | - | - | 0.11 | - | - |

- Between measurement, Observed Power, Repeatability and Intraclass Correlation Coefficient are reliability statistics that apply to all tests

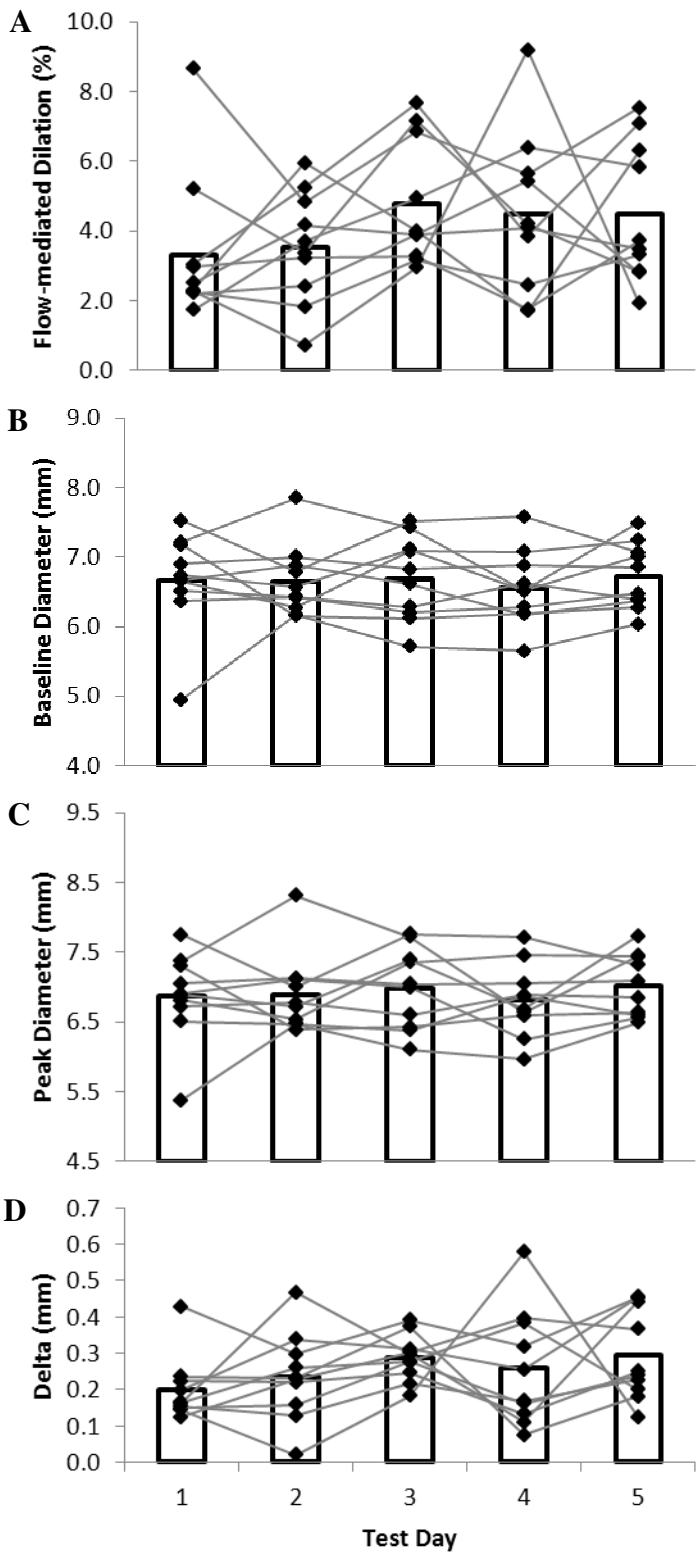



**Figure 2.5** Day-to-day variability of measures between five FMD tests performed on separate days for 10 subjects. Group mean for each test is represented by the bar graph.

**Table 2.4** Statistics for flow-mediated dilation properties associated with five different occlusion pressures.

| | Occlusion Pressure | | | | |
|---|---|---|---|---|---|
| Percent Dilation | 175 mmHg | 200 mmHg | 225 mmHg | 250 mmHg | 300 mmHg |
| Mean | 5.5 | 4.5 | 3.9 | 4.5 | 5.1 |
| SD | 3.2 | 2.5 | 1.1 | 2.5 | 2.3 |
| Coefficient of Variation | 0.57 | 0.55 | 0.28 | 0.55 | 0.45 |
| Between Measurement (*p* value) | - | - | 0.47 | - | - |
| Observed Power | - | - | 0.22 | - | - |
| Repeatability | - | - | 5.78 | - | - |
| Baseline | 175 mmHg | 200 mmHg | 225 mmHg | 250 mmHg | 300 mmHg |
| Mean | 6.6 | 6.5 | 6.5 | 6.5 | 6.6 |
| SD | 0.8 | 0.6 | 0.4 | 0.7 | 0.5 |
| Coefficient of Variation | 0.12 | 0.09 | 0.07 | 0.11 | 0.08 |
| Between Measurement (*p* value) | - | - | 0.99 | - | - |
| Observed Power | - | - | 0.05 | - | - |
| Repeatability | - | - | 1.16 | - | - |
| Peak | 175 mmHg | 200 mmHg | 225 mmHg | 250 mmHg | 300 mmHg |
| Mean | 6.9 | 6.8 | 6.8 | 6.8 | 6.9 |
| SD | 0.8 | 0.6 | 0.5 | 0.7 | 0.5 |
| Coefficient of Variation | 0.12 | 0.09 | 0.07 | 0.10 | 0.07 |
| Between Measurement (*p* value) | - | - | 0.85 | - | - |
| Observed Power | - | - | 0.09 | - | - |
| Repeatability | - | - | 1.25 | - | - |
| Delta | 175 mmHg | 200 mmHg | 225 mmHg | 250 mmHg | 300 mmHg |
| Mean | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 |
| SD | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 |
| Coefficient of Variation | 0.63 | 0.54 | 0.28 | 0.48 | 0.42 |
| Between Measurement (*p* value) | - | - | 0.41 | - | - |
| Observed Power | - | - | 0.24 | - | - |
| Repeatability | - | - | 0.40 | - | - |

- Between measurement, Observed Power and Repeatability are reliability statistics that apply to all tests.

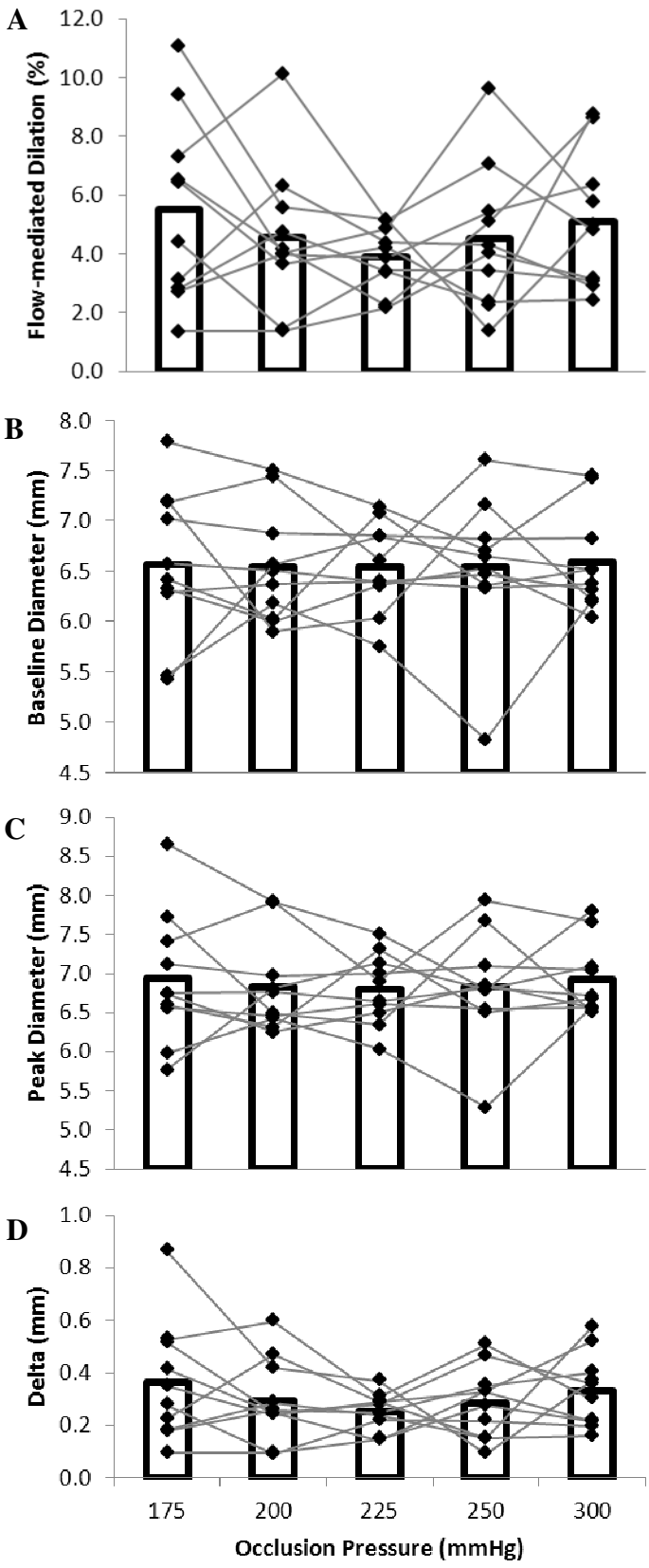



**Figure 2.6** Variability of measures between five FMD tests performed at different occlusion pressures for 10 subjects. Group mean for each test is represented by the bar graph.

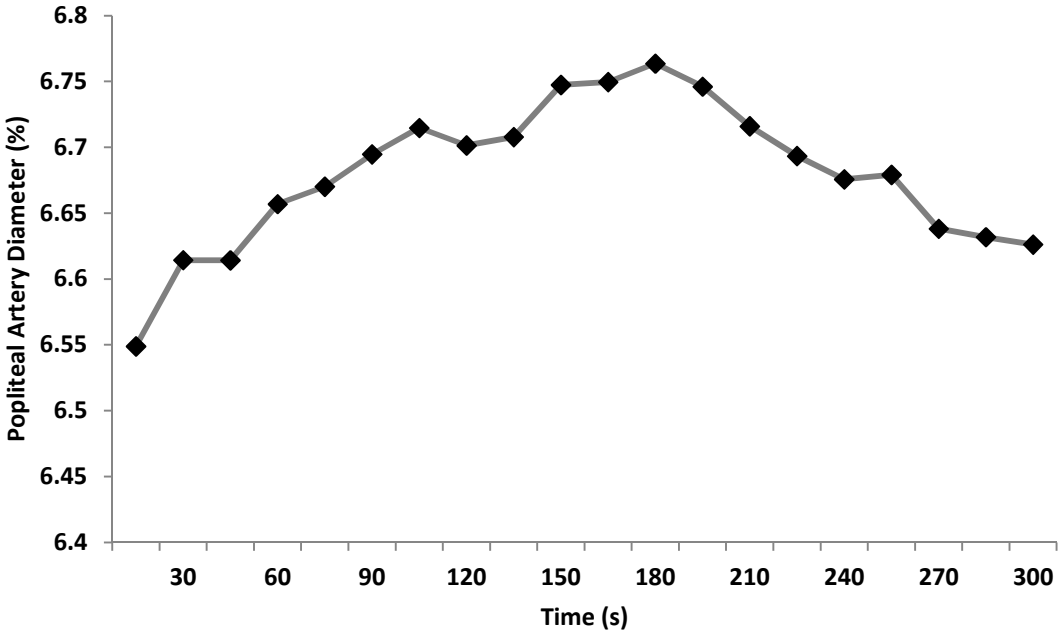



**Figure. 2.7** Time course of popliteal artery dilation following reactive hyperemia. Time course in 15-second intervals of popliteal artery FMD expressed as percent difference from baseline after distal cuff occlusion. Each point on the graph represents an average of all the data available at that time-point.

## 2.4 DISCUSSION

The main goals of this study were to investigate the test-to-test (intraday) and day-to-day (interday) reliability of FMD in the popliteal artery, and to examine the effects of five different occlusion pressures on the dilatory response in the popliteal artery. The main findings were as follows: 1) repeatability values for these tests were large indicating a high systematic error of the technique; 2) reliability of FMD tests was poor both within and between testing days (low ICCs); 3) there was no significant difference between FMD tests performed in the same day, across five days or tests performed at five different occlusion pressures.

Repeatability is used to examine the influence of measurement errors on data analysis and is an indicator of absolute reliability, such that the difference between repeated measurements for the same subject is expected to be less than 2.77 × within subject SD (Sw) for 95% of observations [where 2.77 is derived as ($1.96 \times \sqrt{2}$)] (Bland & Altman, 1996). In the present study, large values of repeatability indicated poor reliability of the technique. Repeatability values were high for intra- and interday FMD, such that a difference of 5.62% and 4.82% respectively would be needed to observe a change in FMD that would not be associated with systemic error. These values for repeatability are as large as the mean FMD measures themselves. This is particularly important for pre- versus post-intervention study designs that use FMD tests as an indicator of endothelial function. With high values of repeatability, the likelihood of detecting a difference is small. The repeatability between days was no worse than the within day measures, suggesting it is measurement error rather than variability in day-to-day physiological responses. Studies in the brachial and radial arteries have also shown poor reliability as estimated by repeatability. Hardie et al. (Hardie *et al.*, 1997) demonstrated that reproducibility of brachial artery FMD was poor and likely to provide inaccurate measurements

for two FMD tests separated by an average of 90 days. Repeatability calculated from reported values for Sw indicated FMD in the brachial artery would need to be approximately 19% to be able to detect changes that could not be attributed to systemic error. This study however, did not use the "present-day" standardized technique, consider age or sex differences in participants and did not control for diurnal variation, recent exercise or caffeine intake. More recently, Brook et al. (Brook *et al.*, 2005) assessed intra- and interday reliability for two FMD tests performed in the same day and two tests performed approximately 7 days apart. The repeatability calculated from reported values for Sw were high for both intraday (10.75%) and interday (10.72%). The authors concluded that radial artery FMD was highly variable. Although this group did control for diurnal variation and dietary intake, they also did not utilize the "present day" standardized technique and did not control for sex differences in participants. Unlike the previous studies, the present study utilized the current standardized technique for FMD tests and controlled for multiple factors.

This study also reported ICC, which provides a measure of relative reliability. Unlike measures of absolute reliability, correlation coefficients are influenced by the range of values measured and give no indication of actual measurement values or systemic variability within the measure itself (Hopkins, 2000). As suggested by Portney and Watkins (Portney & Watkins, 2000), ICC values >0.75 are considered to be reliable. In the present study, ICC values for intraday (0.36) and interday (0.25) reliability of FMD indicated very poor reliability of the technique. These ICC values are similar to those reported by Brook et al (Brook *et al.*, 2005) for intraday (0.38) and interday (0.23) measures analyzed by the same reader. Alternatively, Welsch et al. (Welsch *et al.*, 2002) reported high reliability of two FMD measures in the brachial artery (ICC = 0.92) performed a week apart. A feature of the present study, is that unlike the

previous studies where only two measures were compared, the present study was the first to systematically compare five measures for the interday reliability and three measures for intraday reliability.

Although reliability of FMD was poor for both intra- and interday comparisons, it is interesting to note that in both cases the reliability of baseline and peak diameters (which are used to calculate the change in diameter and then FMD) were much better than that of delta diameter and FMD (expressed as a % change from the baseline value). The repeatability for intraday comparisons was much smaller for baseline (1.92 mm ie. ~30% of the baseline diameter) and peak diameter (2.33 mm ie. ~35% of the peak diameter) compared with delta diameter (0.36 mm ie. greater than 100% of the delta diameter) and FMD (5.62% ie. greater than 100% of the FMD). Additionally, repeatability for interday comparisons was much smaller for baseline (1.00 mm i.e. ~ 15% of the baseline value) and peak diameter (1.32 mm, i.e. ~ 20% of the peak value) compared to delta diameter (0.33 mm, i.e. greater than 100% of the delta) and FMD (4.82% ie. greater than 100% of the FMD). Similarly, ICC values for intra- and interday reliability were much higher for baseline and peak diameter indicating a higher reliability. In the case of intraday reliability, ICC values indicated a good reliability of baseline and peak diameter (0.91 and 0.86, respectively). In agreement with these results, West et al. (West *et al.*, 2004) found baseline diameter to have a small coefficient of variation (CV range of 1-13%), whereas the maximal FMD was associated with a much larger variability (CV range 1-84%). It appears that although baseline and peak diameters can be measured with adequate reliability, the delta diameter is so small that when adjusted to ratios of % change in diameter or FMD, the error is magnified resulting in poor reliability of the measures.

The FMD values reported in the present study are comparable to others in literature (Thijssen *et al.*, 2008, 2011*b*). Thijssen et al. (Thijssen *et al.*, 2008) reported similar values for the popliteal artery of baseline diameter (6.2 ± 1 mm), peak diameter (6.6 ± 1 mm) and FMD (6.1 ± 3.3%). Additionally, the time course of popliteal artery FMD depicted in Figure 2.7 is comparable to the time to peak diameter reported by the same group (181 ± 85 seconds) (Thijssen *et al.*, 2008).

In the present study, there were no significant differences between FMD tests performed on the same day or on different days. Nevertheless it is acknowledged that the study was underpowered to detect these differences and thus there is the possibility of a type II error (stating that there are no differences when in fact there actually are); in fact, for the three within-day tests a p-value of 0.08 approaches significance (further demonstrating variability of the measure). Although we acknowledge this is a limitation in the present study, we were interested in measuring reliability in this sample size as the majority of research focused studies use small sample sizes (often 10 or less subjects) to test physiological adaptations to various exercise protocols, or to examine differences between sexes and clinical populations (Joannides *et al.*, 1995; Mullen *et al.*, 2001; Betik *et al.*, 2004; Green *et al.*, 2006, 2010; Parker *et al.*, 2006, 2011; Pyke & Tschakovsky, 2007; Black *et al.*, 2009; Pyke *et al.*, 2009; Tinken *et al.*, 2010). For this reason we wanted to determine the reliability of the FMD technique in these smaller sample sizes and understand the limitations when applied to these samples. That being said, using computational modelling of day-to-day FMD measures we generated data for an increased sample size, maintaining the same variability in the data. By doubling the sample size (n=20) we doubled the statistical power (0.57), approached significance for differences between tests (p = 0.06), but with very little change in repeatability (4.69%) and ICC (0.201). When the sample

size was increased to 40, statistical power above 0.8 was achieved and there was a significant difference between tests performed on different days ($p = 0.002$). Interestingly, repeatability remained relatively unchanged (4.63% vs 4.82%) compared to the original sample size of 10. Thus, although the present study was not powered to state that there were no statistically significant differences in measures within or between days, it is likely that the repeatability and ICC values (which were the main focus of the study) would not be greatly altered with an increased sample size. The measurement error of the FMD technique with 10 subjects is likely to remain consistent in larger samples.

Another important aspect of this study was to assess the intraday reliability, to determine if a single test was repeatable, and therefore an accurate representation of vasoreactivity in the popliteal artery. By averaging two tests (on each day there was an FMD test at 250 mmHg and one at another occlusion pressure), we reduced measurement error (decreased repeatability from 4.82% to 3.90%) suggesting that multiple measures averaged may provide a more accurate assessment of changes in FMD. Sorensen et al. (Sorensen *et al.*, 1995) noted that the repeatability of the measure could be reduced from 5.2% to 2.6% when the number of FMD tests is increased from one to four for both pre- and post-measures for a given intervention. In the present data we were able to examine the effect of averaging by comparing the average of the three tests within a day and the five tests over five days (at 250 mmHg occlusion pressure). As shown in Figure 2.8, the correlation of the averaged values for FMD was not high ($r = 0.641$). Although significant ($p = 0.046$) there is not a good reliability and there is considerable scatter about the line of identity Therefore, although averaging tests may reduce the repeatability slightly, the reliability of the measure is not greatly improved in this sample. Further analysis is needed to determine the importance of averaging measures of FMD responses.

The standardization of the FMD technique is critical for the comparison of FMD values obtained between different clinicians and research centres. An aspect of the FMD protocol that remains variable is the occlusion pressure used for the five minute cuff occlusion period. As hypothesized, there were no significant differences between FMD tests performed at five different suprasystolic occlusion pressures. In that regard, however, a larger sample size is needed to definitively conclude that there is no effect of occlusion pressure on FMD response in the popliteal artery.

Shear rate (calculated as blood velocity/vessel diameter) is commonly used as a proxy measure of shear stress when viscosity measures cannot be obtained. The shear stress stimulus has been identified as the major contributor to the magnitude of FMD (Pyke & Tschakovsky, 2007). In some studies of FMD the shear stress is reported in order to estimate the dilation per shear rate (Pyke & Tschakovsky, 2005). In the present study, although we were confident in our vessel diameter measures we were not confident in the blood velocity measures which showed large variability. Therefore, it is plausible that the variability in shear stress may affect variability and repeatability of FMD, and understanding this interplay requires further study.

In addition to limitations mentioned above, the present study did not control for dietary intake in the hours leading up to FMD tests. No blood samples were taken and assessed for hematological variables known to influence arterial vasodilation such as; lipids (Vogel *et al.*, 1996; Steinberg *et al.*, 1997); homocysteine (Tawakol *et al.*, 1997; Chambers *et al.*, 1999); fibrinogen (Allen *et al.*, 2000); and blood glucose (Title *et al.*, 2000). Although dietary intake should not account for the poor reliability of FMD tests within a day, it is possible that some of the variability in FMD measures between days could be attributed to changes in hematological factors. Nevertheless, the day-to-day variation was no worse than the within day repeatability.

All ultrasound imaging and analysis were performed by the same investigator. To ensure no bias, analysis should have been completed by a blinded observer. Furthermore, this study was completed with manual analysis of all FMD tests. This restricted the frequency at which images could be selected for diameter measurements. Manually-derived diameter measurements were taken every 15 seconds in the present study. Current edge-detection software programs allow for diameter measurements to be obtained as frequently as each cardiac cycle during the time period of interest. This may provide a more representative time course of vasodilation in the artery being imaged.

In conclusion, this study demonstrated the popliteal artery FMD measure has poor reliability for both test-retest and day-to-day reliability. The lack of reliability using this technique suggests that interpretation of individual or group changes should be made with caution, particularly when the FMD values are used for clinical diagnosis. Baseline and peak diameter measures have stronger reliability; however the change in diameter is so small that the variation in these measures may be magnified when converted to a delta diameter and the FMD ratio, ultimately accounting for the poor reliability exhibited in those measures. Studies involving larger sample sizes are required to confirm this. Even with the ever increasing number of studies addressing reliability of the FMD tests, there remains little consensus. Due to the noninvasive nature of this technique it is likely that it will continue to be a popular research and diagnostic tool for assessing endothelial function. As such, known factors affecting vascular reactivity should be strictly controlled for and studies should adhere to standardized protocol and analysis for this technique.

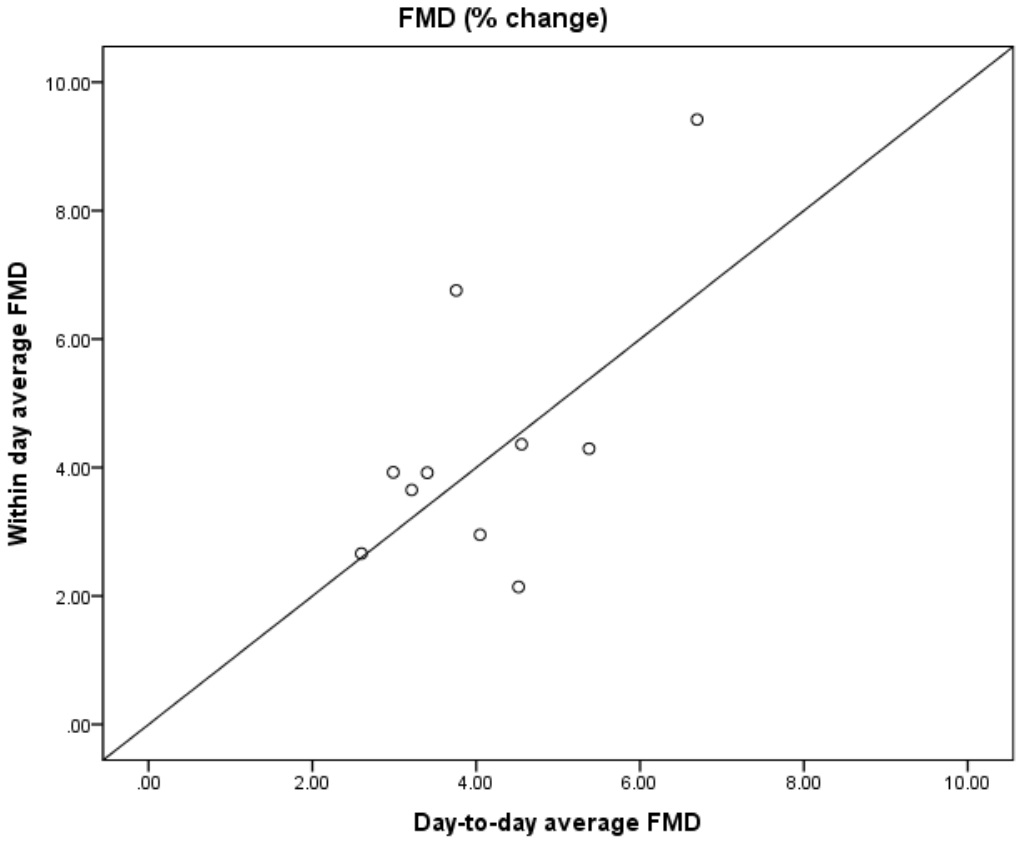



**Figure 2.8** Relationship between the average of three FMD tests within a day and the average of five FMD tests over different days (all at 250 mmHg occlusion pressure) ($r = 0.641^*$). $^*p < 0.05$

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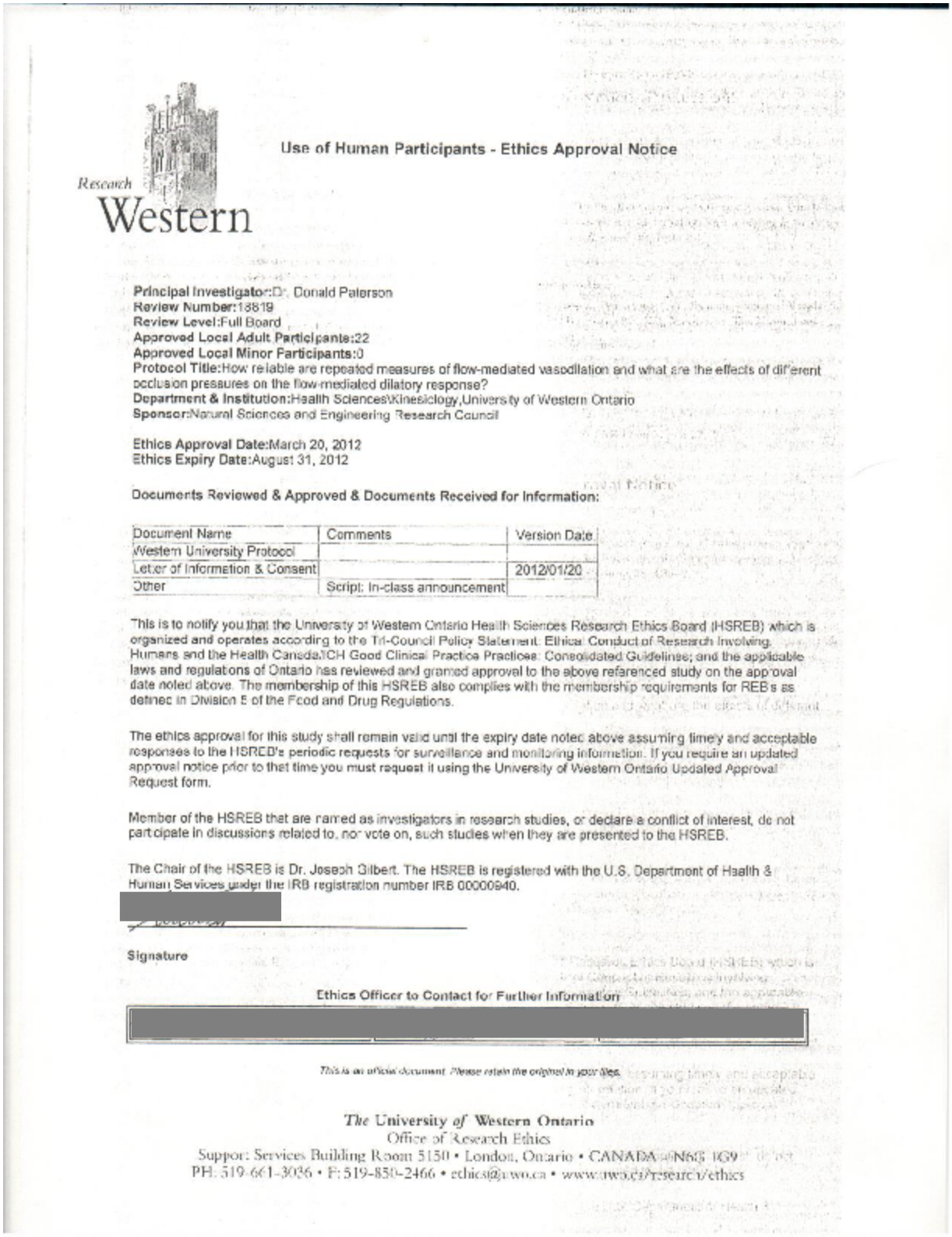
**APPENDIX A**



Research Western

Use of Human Participants - Ethics Approval Notice

**Principal Investigator:**Dr. Donald Paterson
**Review Number:**13819
**Review Level:**Full Board
**Approved Local Adult Participants:**22
**Approved Local Minor Participants:**0
**Protocol Title:**How reliable are repeated measures of flow-mediated vasodilation and what are the effects of different occlusion pressures on the flow-mediated dilatory response?
**Department & Institution:**Health Sciences\Kinesiology,University of Western Ontario
**Sponsor:**Natural Sciences and Engineering Research Council

**Ethics Approval Date:**March 20, 2012
**Ethics Expiry Date:**August 31, 2012

**Documents Reviewed & Approved & Documents Received for Information:**

| Document Name | Comments | Version Date |
|---|---|---|
| Western University Protocol | | |
| Letter of Information & Consent | | 2012/01/20 |
| Other | Script: In-class announcement | |

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[redacted]

Signature

Ethics Officer to Contact for Further Information

[redacted]

*This is an official document. Please retain the original in your files.*

*The* University *of* Western Ontario
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Support Services Building Room 5150 • London, Ontario • CANADA • N6G 1G9
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**CURRICULUM VITAE**

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| **Name:** | **KAITLIN M. MCLAY** |
| **Post-Secondary Education and Degrees:** | The University of Western Ontario<br>London, ON<br>2010-2012 M.Sc.<br>Kinesiology<br><br>University of Guelph<br>Guelph, ON<br>2006-2010 B.Sc. Honours<br>Biological Sciences<br>Minor in Nutritional Sciences |
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**Honours and Awards:**

| | |
|---|---|
| 2011-2012 | Queen Elizabeth II Graduate Scholarship in Science and Technology |
| 2010-2012 | University of Western Ontario Graduate Research Scholarship |
| 2007-2010 | Dean's Honour List |
| 2006 | University of Guelph Entrance Scholarship |
| 2006 | Governor General's Award |
| 2006 | Dr. Keith Hopkinson Memorial Scholarship |

**Abstracts/Presentations:**

SPENCER, M.D., MURIAS, J.M., GRAVELLE, B.M.R., **MCLAY, K.M.**, KOWALCHUK, J.M. & PATERSON, D.H. (2011). Does improved local O2 distribution explain faster VO2 kinetics during smaller compared to larder moderate-intensity transitions? Medicine & Sciences in Sports & Exercise: May 2011. Vol 43(5):387. *Abstract*

**MCLAY, K.M**., DOGRA, S. & PATERSON, D.H. (2012) Responsiveness of the popliteal artery in older trained and untrained women. Medicine & Science in Sports & Exercise: May 2012. Vol 44(5):S411. *Abstract*

**MCLAY, K.M.,** MURIAS, J.M., NEDERVEEN, J. & PATERSON, D.H. (2012). Test-to-test repeatability of FMD measures in healthy young adult males. Applied Physiology, Nutrition, & Metabolism: In print. *Abstract*